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A Review of Serological Tests for the Diagnosis of Hydatid Disease

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Reviewing the literature on hydatid serology for the period 1958–66, the author concludes that the complement-fixation test is not the most sensitive procedure but may have value for postoperative evaluation. The haemagglutination, bentonite-flocculation and latex-agglutination tests are the procedures of choice at present. The fluorescent-antibody test shows much promise, but requires further evaluation. The intradermal test performed with standardized antigen is useful for diagnosis and epidemiological studies. Reliable serological diagnosis of hydatid disease in animals awaits the use of more specific antigen. Although many related species share common antigens with Echinococcus granulosus, *the sensitivity of the diagnostic serological tests is high because of the strongly specific response elicited by the specific antigens used. The need for further standardization of both antigen and test procedure is pointed out.*

The serology of hydatid disease dates from the early use of the complement-fixation (CF) test in the first decade of this century (Ghedini, 1906; Ymaz-Apphatie & Lorentz, 1908; Weinberg & Parvu, 1908). A brief review of the early literature is presented by Hiles (1926). More recent reviews are found in the publications of Powers & Churchill,[2] Kien-Truong (1960), Giunchi (1960), Kent (1963a), and Sorice et al. (1966).

In addition to the intradermal (Casoni) skin test, the serological methods commonly employed for the diagnosis of hydatid disease are the CF test, the haemagglutination (HA) test, the bentonite-flocculation test (BFT) and the latex-agglutination (L) test. An indirect fluorescent-antibody (IFA) method has also been described.

For the purpose of this paper the literature of the past 8 years on human diagnostic techniques has been covered in detail. A section on animal diagnostic techniques in also given. This period marks the introduction of the newer serological methods such as HA, L, BFT and IFA. The sensitivities of these methods for proved cases of hydatid disease are tabulated in Table 1. Since it is not possible to evaluate the clinical status of all the patients reported in the literature and since antibody concentration declines in the body after extirpation or calcification of the cyst (Abou-Daoud & Schwabe, 1964; Sorice et al., 1966) the sensitivity data must be viewed with a certain amount of objectivity. An author who studies patients with calcified cysts will find a lower sensitivity in his tests than one who does not.

The newer serological techniques, such as haemagglutination and flocculation, appear to be more sensitive than the CF test. Once again, one must be careful in making sweeping generalizations because so many variables influence the sensitivities reported in the literature. The test of choice today for the diagnosis of hydatid disease appears to be the haemagglutination test. However, this technique also involves diagnostic problems which must be solved.

COMPLEMENT-FIXATION TEST (Tables 1 and 2)

The sensitivity of the CF test (as reported during the past 8 years) ranges from 36% (Kagan et al., 1959) to 93% (Picciocchi, 1960). An approximate average sensitivity for the publications listed in Table 1 is 69%. A number of attempts to increase the sensitivity of this procedure have involved the use of conglutination (Angelillo et al., 1959; Pautrizel

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[2] Powers, L. E. & Churchill, C.W. (1959). Paper from the American University of Beirut, available only by request to the authors.

TABLE 1

RELATIVE SENSITIVITY OF SEROLOGICAL TESTS FOR THE DIAGNOSIS OF HUMAN HYDATID INFECTIONS

Reference	Year	No. of sera tested	Test sensitivity (%) [a]				
			ID	CF	HA	L	BFT
Bensted & Atkinson	1958	70	87	87			
Monti & Picciocchi	1959	26	70	100			
Garabedian, Matossian & Suidan	1959	79	89	77	87		
Magath	1959	41	87	93			
Norman, Sadun & Allain	1959	18		39			100
Kagan, Allain & Norman	1959	30		36	97		90
Mandras & Addis	1960	34	76	65	79		
González-Castro & Chordi	1960	24		79			96
Rokower	1960	100	78	60			
Picciocchi	1960	30	70	93			
Morellini & Ferri	1960	61	64	92			
Fischman	1960a	51		84	85	92	
Chordi	1962	60	86	62	85		71
Knierim & Niedmann	1961	106		61	85		
Knierim & Niedmann	1962	203		75	85		
Pediatria	1963	45	48	43	74		
Arabatzis & Papapanagiotou	1963	120	74	62	90		
Nobili, Cosentino & Rizzo	1963	30	57	77			
Pauluzzi & Sorice	1963			67			
Cowling	1964	83	81	76			
Zorihina	1964	11			91	86	
Sabar & Onat	1964	18	83	50			
Abou-Daoud	1965	242	65	51	66		
Sorice & Castagnari	1965	28				50	79
Sorice, Pauluzzi, Castagnari & Tolu	1965	42		50	80		
Kagan, Osimani, Varela & Allain	1966	57			67	72	70
Zanussi, Sorice & Castagnari	1966	68		60	88	71	73
Sorice, Castagnari & Tolu	1966			75	98	78	73
Average sensitivity			74	69	84	75	82

[a] The test sensitivity is given as the percentage of serologically positive results in cases of hydatid disease. The various tests are abbreviated as follows: ID = intradermal; CF = complement-fixation; HA = haemagglutination; L = latex-agglutination; BFT = bentonite-flocculation test.

& Bailenger, 1961), of a semi-quantitative micro-method (Pauluzzi, 1964a, 1964b), and of isofixation curves to evaluate antigen concentrations (Pauluzzi & Serra, 1965). The main problems in the use of CF are the choice of technique and the choice of antigen. The Italian workers consider on the whole that sheep hydatid fluid is superior to human or bovine fluids (Pauluzzi, 1964b). Pauluzzi & Sorcie (1963) found the " globulin antigen " of hydatid fluid (Lorenzo, 1960) very reactive. On the other hand, Fischman

(1965) found human hydatid fluid superior to ovine or bovine fluids in his laboratory.

In addition to a lower sensitivity, the CF test appears to have a higher nonspecific reactive rate than agglutination methods. Nonspecific rates as high as 28% (González-Castro & Chordi, 1960) have been reported. Table 2 lists the nonspecific positive

TABLE 2
NONSPECIFIC POSITIVE RESULTS OBTAINED IN THE COMPLEMENT-FIXATION TEST WITH NON-HYDATID SERUM

Reference	Year	Percentage nonspecific positive results
Bensted & Atkinson	1958	0.4
Garabedian et al.	1959	5.9
Knierim	1959	3.8
Mandras & Addis	1960	2.5
González-Castro & Chordi	1960	28.0
Picchiocchi	1960	4.5
Morellini & Ferri	1960	0.4
Knierim & Niedmann	1961	4.2
Chordi	1962	14.0
Arabatzis & Papapanagiotou	1963	2.7
Nobili et al.	1963	6.2
Zanussi et al.	1966	1.3

results obtained with control sera as reported in the publications available to the author. The average nonspecific rate ranged from 1% to 5% with human sera. Vural et al. (1964) attribute these reactions to aggregation of abnormal globulin in serum of individuals without hydatid disease. These globulin aggregates can be absorbed from the sera and react nonspecifically with normal globulin components in hydatid fluid antigen. Kagan et al. (1960) and Gräfe (1964) attributed these nonspecific reactions in the serum of sick individuals to the presence of autoantibodies which react with host protein in the hydatid antigen.

From a diagnostic point of view, the nonspecific reactivity in the CF test of serum from patients with cancer is very important. Bensted & Atkinson (1958) listed one such positive reaction in a cancer patient which they attributed to vaccination with a sheep rabies vaccine. Cowling (1964) reported two false positive reactions in individuals with cancer of the pancreas. Knierim & Niedmann (1961) reported 4 cases, and Gräfe (1964) found 3 out of 5 patients with lung cancer giving false positives in the hydatid CF test. The serum from a patient with a synovial sarcoma was positive in the CF test and negative in the skin test (Meadows & Sage, 1966). Kagan (unpublished) has found at least 9 sera from cancer patients that were strongly positive in the CF test. These sera were all negative by HA.

The CF test result reverts to negative more quickly than agglutination reactions following removal of a hydatid cyst from the body. The technique may, therefore, have value for postoperative evaluation. A persistent CF titre 6–12 months after an operation may indicate the presence of a second cyst (Sorice et al., 1966).

HAEMAGGLUTINATION TEST (Table 3)

The HA test has been finding increasing use in the diagnosis of hydatid disease since its introduction by Garabedian et al. (1957a, 1957b). Most workers who have used this technique find it more sensitive than the CF test (Knierim, 1961). The sensitivity of the technique ranges from 66% to 100%, with an average of approximately 83%. The nonspecific reaction rate is very low, ranging in most instances from 1% to 2%. Zorihina (1964) is the only author to report a nonspecific positive HA test result in a patient with cancer. Kagan et al. (1959), considering a titre of 1:50 or above as a positive test result, reported a nonspecific rate of 14.2% for 246 sera tested. Further investigation showed that the nonspecific reactions were due to host protein in the hydatid fluid antigen, which gave many false positive titres in the range 1:50–1:200. Kagan (1963) suggested that the specificity of the test could be increased by considering a titre of 1:400 or above as positive. On this criterion, only 5 out of 175 sera (2.8%) from patients with other diseases were positive (Kagan, 1965). Titres of 1:400 or greater were considered positive by Arabatzis & Papapanagiotou (1963), Zorihina (1964), Castagnari & Tolu (1964) and Sorice et al. (1965). Knierim & Saavedra (1966) considered titres of 1:200 or greater as positive.

Some of the variables of the test are the type of red blood cells employed, the method of tanning, the antigen used, and the method of reading the test. The microhaemagglutination test lends itself to mass

TABLE 3

SENSITIVITY AND SPECIFICITY OF THE HAEMAGGLUTINATION TEST FOR HUMAN HYDATID DISEASE

Reference	Year	No. tested	Percentage positive	Normal sera		Other diseases		Type of cell used [a]	Antigen [a]	Minimum positive titre
				No. tested	Percentage positive	No. tested	Percentage positive			
Addis & Mandras	1958	34	79							
Garabedian et al.	1959	79	87	105	0				B	1 : 360
Kagan, Allain & Norman	1959	22	97	122	17.2	124	11.3	S	P	1 : 50
Mandras & Addis	1960a	34	79	200	1.0			H	H, B	1 : 80
Garabedian, Malakian, & Matossian	1960	43	88			100	0	H	B	
Zorihina	1961a	55	96			44	2.3			
Knierim & Niedmann	1961	106	85	119	0	58	15.5	H	HHF	1 : 200
Chordi	1962	60	85	39	0	121	4.1	S		1 : 25
Pediatria	1963	45	74							
Arabatzis & Papapanagiotou	1963	120	90	150	2.0			S	HHF	1 : 640
Zorihina	1964	11	91			35	2.8			1 : 640
Abou-Daoud	1965	242	66					O	SHF, B	1 : 40
Sorice, Pauluzzi, Castagnari & Tolu	1965	42	80						SHF, B	1 : 400
Kagan et al.	1966	57	67	22	0	45	0	S	SHF	1 : 400
Castagnari & Tolu	1964	40	85	32	12.5				SHF, B, H, cysts	1 : 400
Chordi et al.	1962	13	92	38	0	75	2.7	S	P, B	1 : 25
Mochmann & Hering	1964	4	100			240	0		SHF, B	
Zanussi et al.	1966	68	88	302	0.3			S	SHF	1 : 400

[a] S = sheep red blood cells; H = human O cells; B = bovine hydatid fluid; P = pig hydatid fluid; HHF = human hydatid fluid; SHF = sheep hydatid fluid.

screening of sera for epidemiological studies. The technique of microtitration for hydatid disease is described by Knierim & Saavedra (1966). A survey of 2889 sera of Brazilian military recruits (Cuadrado & Kagan, unpublished) in which the microhaemagglutination method was used gave excellent correlation with the known epidemiological distribution of hydatid disease in that country.

The use of formolized cells greatly enhances the usefulness of the test. Such cells have been used by Parodi (1960), Mandras & Addis (1960b), Allain & Kagan (1961) and Chordi et al. (1962b). Formolized cells can be frozen or lyophilized and kept for at least 18 months with no loss of activity (Kagan, 1963).

The diagnosis of hydatid lung cysts has been a problem in some laboratories. Most workers report a lower sensitivity with serum from patients with this type of involvement (Garabedian et al., 1959; Picciocchi, 1960; Jonathan, 1960; Knierim & Niedmann, 1961; Arabatzis & Papapanagiotou, 1963). On the other hand, this does not appear to be a problem in some countries where both lung and liver cysts are commonly found. This problem is discussed by Kagan et al. (1966).

BENTONITE-FLOCCULATION AND LATEX-AGGLUTINATION TESTS (Table 4)

The bentonite-flocculation test (BFT) was first used for the study of hydatid disease by Norman et al. (1959). A comparative study of the HA test and the

TABLE 4

SENSITIVITY AND SPECIFICITY OF THE BENTONITE-FLOCCULATION TEST (BFT) AND THE LATEX-AGGLUTINATION (L) TEST FOR THE DIAGNOSIS OF HUMAN HYDATID DISEASE

Reference	Year	Percentage sensitivity [a]		Percentage non-specificity [b]	
		BFT	L	BFT	L
Norman, Sadun & Allain	1959	100		4.5	
Kagan, Allain & Norman	1959	90		0	
Fischman	1960	97	92		
Parodi	1961			0	
González-Castro & Chordi	1962	96		27.8	
Szyfres & Kagan	1963	94		3.0	
Mochmann & Hering	1964		50		0
Zorihina	1964		86		0
Chordi	1964	71			
Sorice & Castagnari	1965	79	50	0	0
González-Castro	1965	88			
Kagan et al.	1966	70	72	7.6	9.1
Sorice et al.	1966		78		0
Zanussi, Sorice & Castagnari	1966	73	71	0	0
Rombert and Fraga de Azevedo	1966	81	76	4.3	0

[a] Percentage of serologically positive results in cases of hydatid disease.

[b] Percentage of serologically positive results in cases without hydatid disease.

BFT (Kagan et al., 1959) indicated that the HA test was slightly more sensitive. Both tests were recommended for diagnostic work. González-Castro (1960), using a slightly different bentonite test (with sensitized particles emulsified in absolute alcohol), found the BFT more sensitive than the CF test. The specificity of the reaction was poor (27% of normal sera reacted). The Italian workers in Sassari report the BFT to be slightly more sensitive than the latex-agglutination (L) test (Sorice & Castagnari, 1965) but not as sensitive as the HA test (Zanussi et al., 1966). Kagan et al. (1960), in an evaluation of 57 hydatid sera, reported the L test to be slightly more sensitive than the BFT. Specificity in their tests with sera from normal individuals was 100%, but with sera from individuals with other diseases approximately 10% reacted in both tests.

The L test was introduced by Fischman (1960a, 1960b), who reported sensitivity as good as obtained in the BFT. Parodi (1961) compared the L test with the CF and HA tests and the BFT, and reported it to be the most sensitive and specific. Szyfres & Kagan (1963) also found the L test as sensitive as HA and BFT with positive sera. Mochmann & Hering (1964) reported that the L test on animal sera gave closer agreement with the CF test than the HA test. Zorihina (1964) found the L test as effective as the HA test and much simpler. Fischman (1965), in an extensive investigation of possible test antigens, reported that human hydatid fluid was much better for the L test than ovine or bovine fluids. He also reported that fresh serum was more sensitive than heated serum; this is supported by the work of Sorice & Castagnari (1965). The latter authors found that titres in both the L test and the BFT tend to drop to zero in 1–2 years after operation. The outstanding work of the Italian workers is reviewed by Sorice et al. (1966).

FLUORESCENT-ANTIBODY TESTS (Table 5)

Fluorescent-antibody tests for the diagnosis of hydatid infection utilize the protoscolices of fertile hydatid cysts as antigen. Excellent sensitivity has been observed in the few published studies. Pozzuoli et al. (1965) conjugated 10 sera from patients with hydatid cysts and all were reactive in a direct test. Panaitesco (1965) was able to circumvent the

TABLE 5

SENSITIVITY AND SPECIFICITY OF FLUORESCENT-ANTIBODY TESTS FOR THE DIAGNOSIS OF HYDATID DISEASE

Reference	Year	Percentage sensitivity [a]	Percentage non-specificity [b]
Fraga de Azevedo & Rombert	1964	100	0
Pozzuoli, Costanzi, Deiana, & Tamburini	1965	100	
Panaitesco, D.	1965	Hooklets and acetabula on scolex fluoresced	
Sorice, Castagnari & Tolu	1966	92	0

[a] Percentage of serologically positive results in cases of hydatid disease.

[b] Percentage of serologically positive results in cases without hydatid disease.

problem of autofluorescence by staining his material with Lugol's solution, which quenched the nonspecific fluorescence. Sorice et al. (1966), using the indirect test, also obtained excellent specificity. This technique shows much promise and should be further evaluated. The use of a preserved antigen would greatly enhance its usefulness in the diagnostic laboratory.

INTRADERMAL TEST (Table 6)

The intradermal test for hydatid disease was introduced by Casoni (1911) and has been extensively employed in all parts of the world. In the 1920s, Australian, Russian and South American workers published extensively on the use of the test. Acceptance of the skin test became more and more widespread up to the late 1930s. In the 1940s, the specificity of antigens for the test was evaluated and many *Taenia* species were found capable of serving as antigen. Evaluation continued into the 1950s (Kien-Truong, 1960).

The use of the intradermal test in epidemiological surveys has given equivocal results (Casley-Smith, 1959; Wolfgang & Poole, 1956; Meltzer et al., 1956). In general, the skin reactivity is high compared with clinical evidence of infection, and a high rate of false positive results was reported. Inspection of Table 6 indicates that false positive rates from 17% to 18% (Bulgakov, 1958; Garabedian et al., 1959; Sorice et al., 1966) up to 45% (Chordi, 1962) have been reported. The reason for this high false positive rate may perhaps be given by the results obtained by Kagan et al. (1966). Using antigen nitrogen contents ranging from 12 μg/ml to 405 μg/ml, these workers found that high antigen concentrations gave many false positive results in control individuals. Thus, with antigen N over 100 μg/ml at least 30%—40% of the controls were positive. The specificity of the test increased as the concentration of antigen nitrogen decreased.

The sensitivity of the intradermal test is good in proven cases, and rates of between 80% and 95% have been reported by a number of workers. The test, however, has not been standardized as regards the amount of fluid injected and the method of reading reactions. The question of the choice of antigen may or may not be important and will be discussed separately. The technique used by Moya & Santamarina (1963) and Kagan et al. (1966) is patterned on the intradermal test for schistosomiasis described in *Snail Control in the Prevention of Bilharziasis* (World Health Organization, 1965). Use of this test should be encouraged with a view to standardization of the method.

Correlation between the intradermal antigen nitrogen content and skin reactivity and specificity has been demonstrated for the schistosomiasis skin test (Kagan et al., 1961) and the fascioliasis skin test (Pautrizel et al., 1962). Correlation of antigen content and specificity in the hydatid skin test was demonstrated by Kagan et al. (1966).

DIAGNOSIS OF HYDATID DISEASE IN ANIMALS (Table 7)

The diagnosis of infection in animal hosts was evaluated by Weinberg & Parvu (1908) and Graetz (1910). These workers found 60% (3 out of 5) of cattle sera positive and 100% (4 out of 4) of pig sera positive by the CF technique (Hiles, 1926). Graetz (1910) reported that normal rabbit sera gave nonspecific positive reactions in the CF test with hydatid fluid. Dennis (1937) tested sheep, and Goddale & Krischner (1930) tested cows. These latter workers found good sensitivity in the intradermal (ID) test (86% of 44 cows positive) but many false positive reactions (11 out of 44 cows positive). The CF test gave positive results in 59% of the infected animals. Turner et al. (1935) evaluated the ID test on dogs and reported it to be very insensitive. Never (1936), Urbain (1936) and Pores (1943) reported the ID test to be of little value with domestic stock. Ljesevic (1955) reported good results in the ID test (85% sensitivity) with animals.

Chordi (1962), González-Castro & Chordi (1961) and Chordi et al. (1962a, 1962b) carried out extensive studies on infected animals. The newer serological methods such as the HA and BF tests are more sensitive than the CF test for the detection of antibody in infected animals. Cross-reactions in cestode infections due to *Cysticercus tenuicollis* and *Coenurus cerebralis* in sheep yielded 10% false positive results. Pinelli (1961) reported 31.9% (15 out of 47) false positives in L tests carried out with cattle and sheep serum. Babos (1962) obtained many cross-reactions with animal sera in CF and precipitin tests. Chordi et al. (1962b) reported a fair sensitivity for antibody in infected dogs with the BF test (5 out of 8 positive) but again about 10% false positive results in non-infected dogs.

The " scoleoprecipitation " test of Šulc & Ismagilova (1962, 1963) can be used with both human and animal sera. Ramazanov (1963) obtained a sensi-

TABLE 6

SENSITIVITY AND SPECIFICITY OF INTRADERMAL TESTS FOR THE DIAGNOSIS OF HUMAN HYDATID DISEASE

Reference	Year	Percentage sensitivity [a]	Percentage non-specificity [b]	Remarks
Petuhov	1957	83.2	6.2	Excellent review of Russian literature. 8/9 positive controls were cancer cases.
Bulgakov	1958	87.4	16.7 [c]	Boiled (1 h) human hydatid fluid was used as antigen. Good review of Russian literature.
Magath	1959	87		
Garabedian et al.	1959	88.6	18.1	0.2 ml injected. Test was very sensitive in lung cyst cases.
Casley-Smith	1959		39.1	In Australian aborigines with no hydatid infection.
Monti & Picciocchi	1959	69.2	29.2	
Bogomolova & Naumovz	1959	87.5	1.6	Antigen was from whole cysts autoclaved in 1 N H_2SO_4, deproteinized and boiled.
Rokower	1960	78.0		Many false positive reactions were obtained.
Giunchi	1960	87.5		
Morellini & Ferri	1960	63.9		Antigen prepared from cuticular membrane and germinal layer of cyst.
Picciocchi	1960	66.7		Cystic membrane antigen used.
Jonathan	1960	85.7		21/22 liver & lung cysts; 7/11 lung cysts; 2/2 spleen cysts. Tests made in Wales.
Zorihina	1961b	91.0	1.6–2.7	ID is most efficient diagnostic test. Specificity reported for unilocular and multilocular cysts.
Davies & Cameron	1961	38.6		In random sample of Canadian Indians.
Arslanova	1961	2.1		Random testing of shepherds with scolices and hydatid fluid antigens.
Osimani & Varela	1962	77.3 23.8	0.6	Pig hydatid fluid used as antigen. Liver cysts gave 77.3 % rate, lung cysts only 23.8 %.
Chordi	1962	86.7	45.4	Positive ID tests observed 5 years following removal of hydatid cysts.
Moya & Santamarina	1963	95.7	0 [d]	Three types of hydatid antigen evaluated, namely, 2 of Fontana's freezing antigens, and crude whole fluid.
Arabatzis & Papapanagiotou	1963	74.2		ID and HA tests detected 92 % of all infected individuals, 3 positive ID tests in patients with cancer.
Kent	1963		4.7	Used fractionated antigen.
Nobili et al.	1963	56.7		
Cowling	1964	77.8	3.1	Diagnosis of lung cysts was less sensitive than liver cysts.
Sabar & Onat	1964	66.7		Lung cysts.
Abou-Daoud	1965	64.6		ID equal in sensitivity to HA reaction. Brain cyst gave negative ID tests.
Sorice, Pauluzzi & Castagnari	1965	83.5 [e]	18.3	
Brand	1965			Recommends scratch tests as more specific than wheal test.
Kagan et al.	1966	72.1	3.3	Review of literature.

[a] Percentage of serologically positive results in cases of hydatid disease.
[b] Percentage of serologically positive results in cases without hydatid disease.
[c] For persons in good health. Patients with other diseases gave a rate of 3.1 %.
[d] For persons in good health. Patients with other diseases gave a rate of 22.2 %.
[e] Immediate skin test (after 15 min). Delayed skin test rate was 76.7 %.

TABLE 7

THE DIAGNOSIS OF HYDATID DISEASE IN INFECTED ANIMALS

Reference	Year	Species tested	Tests	Test results	
				Percentage sensitivity [a]	Percentage nonspecificity [b]
Angelillo, Corticelli & Masia	1959	Cattle	Conglutination	62.9	9.2
Norman, Sadun & Allain	1959	Wild animals	BFT, CF	4.0	0.5
Olteanu	1960	Goats, cattle	ID	70–90	
González-Castro & Chordi	1961	Sheep	BFT	73 [c]	0
Pérez-Fontana	1961	Immunized dogs and sheep	" Lysis "	Lysed hydatid scolices	Cross-reacted with cestode infections due to *Cysticercus tenuicollis* and *Coenurus cerebralis*
Pinelli	1961	Cattle, sheep	HA L	75.7	40.5
Chordi	1962	Sheep	CF BFT HA	27 72 80	
Chordi, González-Castro, Tormo & Diaz	1962	Sheep	HA	80.0 [d]	0
Chordi, González-Castro & Tormo	1962	Dogs	CF BFT	37.5 62.5	5.5 8.3 [e]
Šulc & Ismagilova	1962	Human, sheep	" Scolexoprecipitation "	All sera positive. Test recommended for hydatid disease diagnosis	
Niculescu et al.	1962	Cattle	ID	71–90	
Chordi	1962	Sheep	CF BFT HA	62 71 85	
Babos	1962	Sheep	CF, Precipitin	Cross-reactions in CF test with animals infected with *Cysticercus pisiformis*. CFT positive first 10 weeks of infection	
Babos & Nemeth	1962	Dogs	Agar-diffusion	Diffusion was not satisfactory. Intradermal test was more sensitive. Antigen from faeces of 14 infected dogs gave positive precipitin tests with immune rabbit serum. Cross-reacted in 7 cases of *T. saginata* infection	
Ramazanov	1963	Sheep Horses	" Scolexoprecipitin "	80.6 91.7	 0
Vibe	1963	Sheep Cattle	ID, HA	82.7 } [f] 84.2 }	
Mochmann & Hering	1964	Sheep	CF HA L Precipitin	5.8 34.6 53.8 6.3	
Pauluzzi & Castagnari	1965	Sheep Cattle Swine	CF, HA	88 — [g] — [g]	21 — [g] — [g]
				CF negative in all animal sera. IHA human and ovine cyst fluid not good. Sheep fluid used as antigen.	
Evranova & Jashina	1965	Sheep	HA, ID, Scolexoprecipitin	ID and HA test gave positive reactions 5–19 days following experimental infection	

[a] Percentage of serologically positive results in cases of hydatid disease.
[b] Percentage of serologically positive results in cases without hydatid disease.
[c] Rate for infected sheep; rate for sheep with other parasities was 10 %.
[d] Other helminths 8.2 %.
[e] Wormy dogs.
[f] Positive 15th day after infection. HA positive 1–18 months following experimental infection in 14 sheep.
[g] Results inconclusive with both antigens.

tivity of 80.6% (25 out of 31) with sheep sera and 92% (33 out of 36) with sera of infected horses. Pérez Fontana (1961) reported a lytic action on hydatid scolices of sera of dogs and sheep immunized with hydatid vaccine.

Babos & Nemeth (1962), using an extract of faeces of infected dogs, reported positive precipitin tests with immune rabbit sera. However, the immune rabbit serum cross-reacted with antigens of *Taenia saginata*.

Vibe (1963) and Babos (1962) found that serological test results became positive 4–10 weeks after infection and remained positive for 18 months. Pauluzzi & Castagnari (1965) found that the CF test gave negative results with sheep, cattle and swine, whereas the HA test was positive with 88% of the infected animals. A nonspecific reaction rate of 21% was obtained.

In summary, serological tests on animal sera are not very sensitive and tend to give a high level of false positive results due to cross-reactions with related cestode infections. The ID test has been employed by several workers (Goddale & Kirschner, 1930; Olteanu, 1960; Niculescu et al., 1962) with relatively high sensitivity (70%–90%). This technique, in the light of recent information on the standardization of the test, requires further evaluation.

OTHER TESTS

Older methods such as the precipitin, miostagmin and Abderhalden tests are no longer considered adequate (Giunchi, 1960). The agar-gel diffusion test (Magath, 1959; Novoselska-Teoharova, 1964) has been evaluated in the writer's laboratory; only a few sera of very high serological titre gave precipitin lines (Chordi & Kagan, 1964).

The eosinophilia test, a diagnostic tool of long standing, has been shown to be a very insensitive method for the diagnosis of hydatid disease (Giunchi, 1960). Rokower (1960) reports that only 25% of 100 patients had eosinophilias greater than 7%. Nobili et al. (1963) found eosinophilia in only 13% of 30 proven cases. Other workers (Monti & Picciocchi, 1959; Cowling, 1964; Giunchi, 1960; Bulgakov, 1958) have reported disappointing results with this method of diagnosis.

The technique of choice today for the diagnosis of hydatid infection appears to be one of the agglutination tests, or the intradermal test; fluorescent antibody methods also show promise. A combination of the haemagglutination and bentonite-flocculation tests gives excellent sensitivity (Kagan, 1963); Sorice et al., 1966).

SEROLOGICAL CROSS-REACTIONS

Antigens prepared from *Echinococcus granulosus* or *E. multilocularis* have many components in common with those of other helminths. Biguet et al. (1962) were able to demonstrate common antigens in hydatid fluid and a number of helminth species by immuno-electrophoresis. Of 9 antigenic components observed in the hydatid antigen, two were also found in *T. saginata*. Bands of identity were also found with *Schistosoma mansoni*, *Fasciola hepatica*, *Onchocerca volvulus*, *Ascaris lumbricoides*, and *Trichinella spiralis*. Kagan (unpublished) obtained good specificity with sheep hydatid fluid antigen and found that this antigen has 1 band in common with *Angiostrongylus cantonensis* antigen and 3 with *T. saginata*. *T. saginata* antigen, however, cross-reacted with antisera to *S. mansoni* (3 bands) and *Diphyllobothrium latum* (1 band).

In the intradermal test, antigens prepared from the following species have been reported as suitable for the diagnosis of hydatid disease: *T. saginata* (Fairley et al., 1929; Núñez & Lopez, 1933; Brisou, 1946; Culbertson & Rose, 1941; Rosas Costa, 1952; Pautrizel & Bailenger, 1961); *T. pisiformis* (Rose & Culbertson, 1939); *T. serrata* (Culbertson & Rose, 1941); *T. crassicolis* (Culbertson & Rose, 1941); *T. taeniaeformis* (Rose & Culbertson, 1940); *Hymenolepis fraterna* (Culbertson & Rose, 1941); *H. nana* (Brisou, 1946); *Diphyllobothrium canis* (Brisou, 1946); *Cysticercus tenuicollis* (Morenas, 1932); *Multiceps serialis* (Pautrizel, 1948; Pautrizel & Serreau, 1947); *Moniezia expansa* (Culbertson & Rose, 1941); *Raillietina cesticillus* (Culbertson & Rose, 1941); and *Diphyllobothrium mansonoides* (Culbertson & Rose, 1941).

Antigens prepared from the following species have been reported as suitable for serological tests on sera from hydatid patients: *Taenia* species in CF tests (Núñez & Lopez, 1933; Rose & Culbertson, 1940; Angel Etcheverry, 1940; Jerioranska & Dobrowalska, 1957; Rosas Costa, 1952; Hariri et al., 1965); *Cysticercus cellulosae* in CF tests (Weinberg, 1909); *A. lumbricoides*, *A. suum*, and *T. spiralis* with sera of immunized rabbits (Jerioranska & Dobrowalska, 1957).

Echinococcus antigens have been reported as suitable for serological tests for the following infections: *C. fasciolaris* in rats (Chung & T'ung, 1939); *C. cellulosae* in man (Greval et al., 1941); and *Taenia* infections in man (Weinberg, 1909).

While a lack of specificity has been shown by certain serological or skin tests because hydatid antigens share common components with other helminth antibodies, and can give cross-reactions with them, published results suggest that the specificity in hydatid serology can be kept within acceptable limits and is in some instances excellent.

There are a number of ways of achieving a high specificity in hydatid serology. The use of dialysis in the preparation of hydatid antigens is one. Dialysed antigens lose many nonspecific components which may interfere in the test or cause cross-reactions. Lemaire & Thiodet (1926) made the observation that serological tests with dialysed hydatid-fluid antigen were more specific than tests with crude whole antigen.

The use of antigenic fractions obtained in other ways may improve specificity. A number of reports deal with the specificity of carbohydrate antigens (Brisou, 1946; Čmelik, 1952; Pirosky et al., 1941; Culbertson & Rose, 1941) and protein antigens (Kent, 1963; Kagan, 1963). The use of diluted antigen may increase the specificity. Chung & T'ung (1939) reported that full-strength antigen gave false positive reactions, whereas diluted antigens were more specific.

The test method and the minimum titre considered as positive are also important factors in determining the specificity. In the haemagglutination test, for example, the specificity would be very poor if titres below 1:400 were considered as indicating diagnostically positive reactions (Kagan, 1963; Sorice et al., 1966). A combination of two serological reactions such as haemagglutination and flocculation increases the specificity of diagnostic tests (Kagan, 1963; Sorice et al., 1966).

A number of suggestions have been made to account for the nonspecificity of certain tests reported in the literature. Kagan et al. (1960) and Gräfe (1964) attribute this to the interaction of auto-antibodies with host components in the hydatid-fluid antigen. The presence of host components in hydatid-fluid antigen was demonstrated by agar-gel analysis by Kagan & Norman (1961, 1963) and Norman & Kagan (1966). Fortunately, such reactions are generally very weak in the haemagglutination test and do not interfere with the sensitivity of the test as a diagnostic tool (Kagan et al., 1960).

The nonspecific response of intradermal skin reactions in patients with carcinomas is attributed by Norris (1965) to interaction of blood-group-P substance in hydatid fluid with anti-P_1 antibody in sensitized patients. The strong cross-reaction in the complement-fixation test with hydatid antigen and sera from patients with carcinomas (Cowling, 1964; Gräfe, 1964) may also be due to the interaction of substances of certain blood groups in the hydatid antigen. Adjustment of intradermal antigen to contain sufficient hydatid reactive nitrogenous material for high sensitivity bypasses the nonspecific results obtained with undiluted cyst fluid which is so commonly used in all parts of the world.

The greatest boon to the diagnostician in hydatid serology is the fact that the antigen-antibody reaction in acute and active hydatid disease is strong. This is probably the main reason for good serological specificity, since cross-reacting systems may be recognized by virtue of their low-level reactivity. The diagnosis of hydatid disease in patients with calcified cysts is more difficult, and when the cysts are in the lungs false negative results are often obtained. The reason for this may not be the test system but the absence of precipitating antibody in the infected host.

Intradermal antigens commonly consist of sterile cyst fluid, usually of human origin. Such antigens contain large amounts of nitrogenous materials giving nonspecific reactions and their use has generally led to poor specificity for epidemiological or diagnostic purposes. Fractionation has produced more specific antigens (Kent, 1963). Dilution of the antigen to a point where specificity is high and nonspecific response is at a minimum is recommended. For *E. multilocularis* antigens the optimum range of nitrogen concentrations in the dialysed antigen is approximately 10 μg/ml – 25 μg/ml (Kagan et al., 1966). The proper nitrogen level for human hydatid-cyst fluid of *E. granulosus* has not been evaluated; it should not differ significantly from that for the *E. multilocularis* antigen.

Summing up, we may state that while *Echinococcus* antigens share many antigenic components with related helminth species, the specificity of hydatid serology can be kept within acceptable limits because nonspecific reactions can readily be recognized by their low reactivity. The sensitivity is good because the hydatid antigens usually elicit a strong serological response in the infected host.

SUGGESTIONS FOR FURTHER RESEARCH

The serology of hydatid disease can be made more meaningful by a concerted effort to standardize methods. The production of several reference control

sera of known antibody nitrogen content from unequivocally proven cases involving various organs of the body (liver, lung, etc.). from relatively acute (young individuals) and chronic cases, would be very helpful; there is sufficient interest to make such a collection of sera useful. Diagnostic laboratories could then standardize their antigens and methods. After sufficient laboratories have tested the sera, mean titres could be determined for the reference sera.

However, it may be premature to attempt the standardization of test procedures at the present time. In an evaluation of a reference collection each participating laboratory should be requested to submit a detailed protocol of its method and the antigen employed for each test. Evaluation of the results and the protocols submitted may indicate the direction for further work.

The intradermal test should be studied further with antigens of known nitrogen or protein content by methods similar to those described by the World Health Organization (1965) for other helminth infections. Sufficient progress has been made in this area to justify such an evaluation.

RÉSUMÉ

Après avoir passé en revue la littérature consacrée, entre 1958 et 1966, au diagnostic sérologique de l'hydatidose, l'auteur formule un certain nombre de conclusions.

L'épreuve de fixation du complément n'est pas très sensible et son taux de non-spécificité est plus élevé que celui des réactions d'agglutination. Elle offre cependant un intérêt lors des investigations postopératoires car elle se négative plus rapidement que les autres réactions après l'extirpation chirurgicale d'un kyste hydatique. L'épreuve d'hémagglutination est de plus en plus employée depuis son introduction en 1957. Elle est sensible et les réactions non spécifiques sont très rares (1 à 2%). Certaines améliorations techniques ont encore accru son utilité. Les épreuves de floculation à la bentonite et d'agglutination au latex donnent de bons résultats. La méthode des anticorps fluorescents fait preuve d'une sensibilité et d'une spécificité très prometteuses; on utilise comme antigène des protoscolex de kyste hydatique. L'intradermo-réaction a été largement employée depuis 1911, notamment lors des enquêtes épidémiologiques. On observe malheureusement une proportion non négligeable de réactions faussement positives. Le choix de l'antigène a une grande importance.

En ce qui regarde le diagnostic sérologique de l'hydatidose chez l'animal, les tests actuels ne sont pas très sensibles. Les infections par des cestodes apparentés à *Echinococcus granulosus* entraînent des réactions croisées et l'on note un pourcentage élevé de réactions faussement positives. L'intradermo-réaction, employée par plusieurs auteurs avec des résultats satisfaisants, doit faire l'objet de nouvelles recherches. De nombreuses autres épreuves ont été abandonnées en raison de leur manque de sensibilité et de spécificité. Le choix se porte actuellement sur l'une des épreuves d'agglutination et sur l'intradermo-réaction. Le recours simultané aux épreuves d'hémagglutination et de floculation à la bentonite donne d'excellents résultats.

L'étude des réactions croisées constatées lors de l'application des techniques sérologiques au diagnostic de l'hydatidose montre qu'*E. granulosus* et de nombreux helminthes apparentés possèdent certains composants antigéniques communs. La spécificité des méthodes sérologiques dans l'hydatidose est cependant acceptable car les réponses non spécifiques sont rapidement identifiées par leur faible titre. Leur sensibilité est satisfaisante, les antigènes hydatiques suscitant généralement une réponse immunitaire forte chez l'hôte infecté.

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